Concanavalin A (with A)- Biotin

Introduction

Concanavalin A, Con A, derived from Canavalia ensiformis (Concanavalin), is a plant lectin protein (Mw = 104 kDa), each subunit can bind a Ca^{2+} and a Mn^{2+}, contains a sugar-binding site, and when the metal ions bind to Con A, it can bind various glycoproteins, glycolipids and α-D-glucose and a-D-mannose moieties in sugars, Biotin, on the other hand, is a small molecule of water-soluble vitamins that has a very strong affinity for avidin. Concanavalin A-Biotin is a labeled protein that combines Concanavalin A and Biotin, and is widely used in biological research, particularly in immunohistochemistry (IHC), immunocytochemistry (ICC), and enzyme-linked immunosorbent assay (ELISA) to detect and quantify biomolecules containing specific sugar structures.

Protocol

Lectins are used to detect glycoproteins in histochemistry, ELISA, and Western blot applications. Sample testing is based on the detection of glycoproteins in tissue sections, adsorbed on microdrop plates, or transferred from electrophoresis gels to nitrocellulose or PVDF membranes.

- Histochemistry Test
  - a. Sample Processing:
  - ➢ Staining of paraffin sections: Deparaffinization and hydration of tissue sections by xylene, or treatment with other scavengers and gradient ethanol, rinsed with tap water for 5 min. If needed, antigen retrieval solution restores the antigen.
  - ➢ Frozen section staining: air-dried sections. Fix sections with acetone immediately before staining. Transfer the slice to the buffer. If endogenous enzyme activity is present, inactivate with appropriate methods
  - b. Optionally, streptavidin/biotin blocking of the sample (e.g., using glycoproteins containing terminal mannose residues) to remove interferences, especially for Con A conjugated Biotin. The avidin Avidin/Biotin Blocking Kit is not recommended, especially when mannose-specific lectins are used.
  - c. Incubate with blocking solution (carbohydrate-free blocking solution, no glycoprotein) for 30 min at room temperature to block non-specific binding. Aspirate excess clogging solution from the slices.
  - d. Add approximately 2-20 μg/mL of Con A-Biotin in PBS (10 mM sodium phosphate, 150 mM NaCl, pH 7.4) and incubate for 30 min at room temperature. Wash with TPBS (PBS + 0.05% Tween® 20).
  - e. Prepare the enzymatic system: Apply ABC-HRP (streptavidin-HPR) or ABC-AP (streptavidin-AP) reagent to the sections and incubate for 30 minutes at room temperature. Wash with TPBS.
  - f. Substrate assays: DAB is recommended for ABC-HRP and BCIP/NBT is recommended for ABC-AP. Rinse with tap water afterwards.

g. Detect the sample.

- ELISA assay

a. Place 50-200 μL of approximately 3 μg/ml glycoprotein solution into the desired well to adsorb the protein of interest onto the microdrop plate. Some wells are not treated and serve as a negative control. Incubate at 37 °C for 1 h. Wash the wells 3 times with TPBS (PBS + 0.05% Tween 20).

b. Incubate with blocking solution (carbohydrate-free blocking solution, no glycoprotein) for 30 min at room temperature to block non-specific binding. Wash three times with TPBS.

c. Add 50–200 μL of approximately 2–20 μg/mL of Con A-Biotin to PBS and incubate for 30 minutes at room temperature. Wash three times with TPBS.

d. Prepare the enzymatic system: Place the ABC-HRP (streptavidin-HPR) or ABC-AP (streptavidin-AP) reagent in the wells (the specific amount is operated according to the instructions) and incubate for 30 minutes at room temperature. Wash with TPBS.

e. Substrate detection: Add a suitable substrate for detection, such as ABC-HRP, it is recommended to choose TMB.

f. Quantitative detection: Spectrophotometric quantification of colored reaction products.

- Western Blot

a. Perform electrophoresis according to standard procedures and transfer proteins to membranes, such as NC/PVDF membranes.

b. Incubate with blocking solution (carbohydrate-free blocking solution, no glycoprotein) for 30 min at room temperature to block non-specific binding. Use enough volume to completely cover the film.

c. Incubate the membrane in PBS containing approximately 2-20 μg/mLCon A-Biotin for 30 min at room temperature. Wash with TPBS (PBS + 0.05% Tween 20).

d. Prepare the enzymatic system: Incubate the membrane with ABC-HRP (streptavidin-HPR) or ABC-AP (streptavidin-AP) reagent for 30 minutes at room temperature. Wash with TPBS.

e. Substrate detection: Add a suitable substrate for detection, DAB is recommended for ABC-HRP, and BCIP/NBT is recommended for ABC-AP. Rinse with tap water afterwards.

f. Detect the sample.

Note

1. Fromulation: 10 mM HEPES, 0.15 M NaCl, pH 7.5, 0.01 mM MnCl2, 0.1 mM CaCl2, 0.08% sodium azide, 10 mM alpha methylmannoside.
2. Product concentration: 5 mg/mL.
3. Recommended concentration: WB: 1:500-1:2000, ELISA: 1:500-1:2500, IHC: 1:250-1:1000, the optimal dilution factor and concentration should be determined by the researcher.
4. Storage and shipping conditions: 6 months at 4°C, blue ice shipping.
5. At neutral and basic pH, Con A exists as a tetramer of four identical subunits; Below pH 5.6, Con A dissociates

into an active dimer of 52 kDa. Acetylation, succinylation, or other derivatization can also produce a stable form with a dimer structure.

6. Calcium or manganese ions are required for all four sugar-binding sites of Con A. Although these divalent metal ions are tightly bound to the peptide structure, buffers that bind calcium should generally be avoided when diluting Con A, as they may gradually lose their activity.

7. This product is for scientific research users only.

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